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LIGHT-SCATTERING AND SPECIFIC REFRACTIVE INCREMENT BEHAVIOR OF AMYLOSE AND DEXTRAN IN METHYL SULFOXIDE–WATER*†

FREDERICK R. DINTZIS AND ROBERT TOBIN
Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture‡, Peoria, Illinois 61604 (U. S. A.)

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ABSTRACT

Light-scattering measurements on polysaccharides in methyl sulfoxide or methyl sulfoxide–water are most reliable when made on a dialyzed solution–solvent system and when proper technique is used to remove the "micro gel" that seems unavoidably present when dried polysaccharide is dissolved in solvent. Discrepancies in published molecular weight–intrinsic viscosity relationships for amylose in methyl sulfoxide appear to be caused in part by differences in the preparation and treatment of samples and possibly by differences in botanical source. The specific refractive increment of dextran is not a linear function of solvent composition in methyl sulfoxide–water. Measurements at osmotic equilibrium indicate that, over a moderate range of composition in methyl sulfoxide–water, dextran preferentially associates with about 2.5 molecules of water per glucopyranosyl residue of the polymer. Effects of long-term storage, sample treatment, and botanical source upon the specific rotation of amylose are examined.

INTRODUCTION

This study was originally planned to find a better solvent than water for light-scattering studies on amyloses. The intention was to avoid the aggregation (retrogradation) of amylose that frequently occurs in aqueous solution. Methyl sulfoxide was chosen, because it is recognized as having excellent solvent properties for polysaccharides and because several studies have been done with Me_2SO as solvent[2–7] to obtain information about the hydrodynamic properties and macromolecular conformations of amylose. However, results of such investigations have led to conflicting interpretations of data, partly because the data themselves have not been consistent. Relationships between intrinsic viscosities, $[\eta]$, of amylose in Me_2SO and

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[*] Dedicated to Dr. Allene Jeanes on the occasion of her retirement.

[†] For a preliminary report, see ref. 1.

[‡] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

molecular weights, $\overline{M}_w$, by light scattering as expressed in Mark–Houwink–Sakurada equations reported by different investigators, seemed to be well outside experimental error. This situation prompted us to examine $\overline{M}_w$ values of amylose in both water and methyl sulfoxide, and $[\eta]$ values in methyl sulfoxide.

Difficulties encountered with amylose led to expansion of the studies to include effects of treatment upon specific rotation and $\overline{M}_w$, and effects of age upon specific rotation. Concern about proper values of the specific refractive increment, $d\bar{n}/dc$, required us to examine such measurements as a function of solvent composition for amylose and dextran. Dialysis experiments undertaken to determine $d\bar{n}/dc$ values in the mixed solvent system, Me_2SO–H_2O, in turn, led to a study of preferential binding of water by dextran.

We conclude that light-scattering measurements on polysaccharides in methyl sulfoxide must be made with great care and should be conducted on dialyzed solution–solvent pairs. Some reasons for discrepancies in $\overline{M}_w:[\eta]$ relationships for amylose in methyl sulfoxide are suggested by this study.

EXPERIMENTAL

Materials. — Samples of dent corn and potato amylose were prepared from appropriate starches at this laboratory by the Schoch[8] procedure and were recrystallized at least twice as the butanol complex. Corn amylose and one batch of potato amylose came from parent samples that had been stored for at least 8 years at ambient laboratory temperatures as air-dried powders.

Amylose fractions were prepared by ethanol precipitation from methyl sulfoxide solution[9]. Precipitated material was washed several times with ethanol and either dried overnight in a hood airstream and stored as a powder or redissolved in methyl sulfoxide and stored as a frozen solution. New amylose fractions were prepared and stored as air-dried powders.

Purified, dry, "soluble" samples were prepared by dissolving the material in boiling, distilled water, clarifying the solution in a preparative ultracentrifuge, and precipitating the product with ethanol. The ethanol complex was washed with ethanol and then several times with dry diethyl ether, and dried overnight in a vacuum oven as described by Fujii *et al.*[2], except that a drying temperature of 90° was used. Samples were then transferred into a closed weighing bottle with minimum exposure to moisture, and stored in a desiccator. Polysaccharide treated in this way was dissolved in methyl sulfoxide on a dry-weight basis for determination of the $[\alpha]_D^{25}$ value.

A laboratory-prepared, clinical fraction of dextran ($\overline{M}_w$ 69,000), produced from *Leuconostoc mesenteroides* NRRL B-512(F), was used in a few experiments; a dextran fraction, T-500, Lot No. 3207, was purchased from Pharmacia. Animal-liver glycogen was purchased from Pfanstiehl Company and may have been a different lot than previously used[7]. Drum-lot methyl sulfoxide from Crown Zellerbach was redistilled under vacuum.

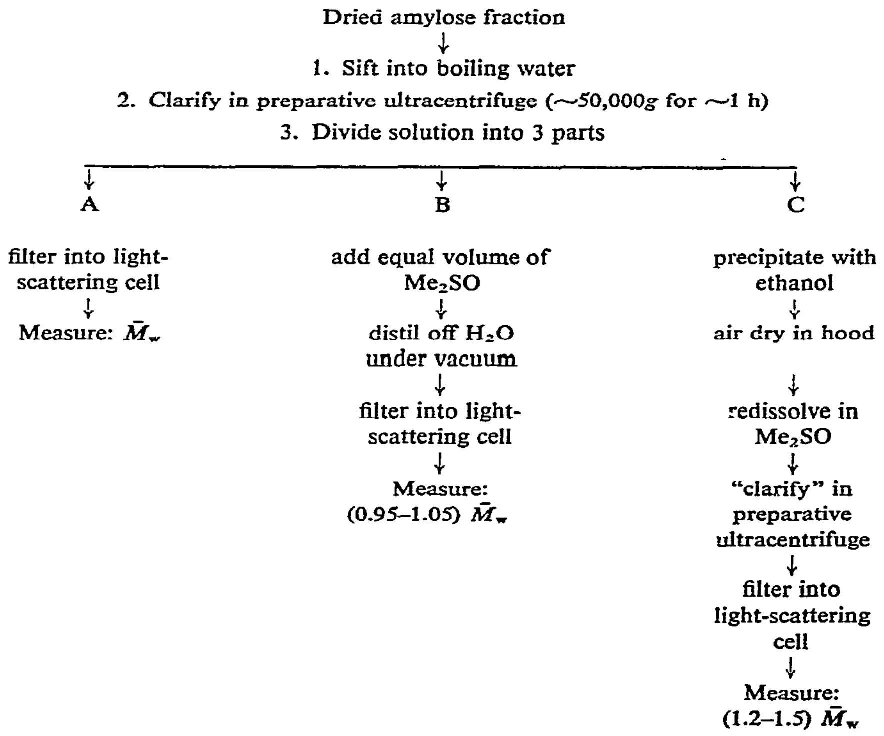
Light-scattering measurements. — These were performed in a previously described[10], modified, Phoenix–Brice instrument which was calibrated at 25° by accepting a value of 46.5×10^{-6} for the Rayleigh ratio of benzene at 436 nm. A disymmetry cell was used in which light intensities were measured at angles of 45, 90, and 135° from the incident beam. Measurements of specific, refractive increment were taken at 25.0° at 546 nm in a Debye type instrument calibrated with potassium chloride by using Kruis' values[11] for $\Delta n/c$ as reference. Solutions were dialyzed in either regenerated cellulose tubing or porous, Vycor® glass thimbles. At high concentrations of methyl sulfoxide, it was found preferable to use glass as a dialysis membrane, because carbohydrate is leached from regenerated cellulose. Intrinsic viscosities at 25° were calculated from relative viscosities obtained with Cannon SemiMicro viscometers, size 75, or a Cannon–Ubbelohde, four-bulb shear-dilution viscometer, size 50. Amylose solutions for determination of $[\eta]$ in methyl sulfoxide were obtained from clarified, aqueous solutions prepared for light scattering by: (*a*) precipitating solute to obtain amylose and redissolving the dry sample in methyl sulfoxide, (*b*) adding methyl sulfoxide to an aliquot of aqueous solution and distilling off water under vacuum, or (*c*) adding methyl sulfoxide to an aliquot of aqueous solution and then dialyzing against 95% Me_2SO–5% H_2O in a bag of regenerated cellulose.

Solutions of amylose in water were prepared for light scattering by sifting dried powder through a 40-mesh sieve and adding the amylose slowly to boiling, distilled water. Amylose solutions were then clarified and processed rapidly to minimize retrogradation. Dextran solutions in water were prepared by using distilled water and stirring for 24 h. These solutions, about 0.5–0.7% w/v, were centrifuged at room temperature in a Beckman model L, preparative ultracentrifuge at 50,000*g* for about 1 h and filtered through a Selas 02 filter directly into a light-scattering cell. Loss of solute, as determined by optical rotation before and after centrifugation, amounted to 8–20% of the material initially dispersed.

Solutions of amylose in methyl sulfoxide or 9:1 (v/v) Me_2SO–H_2O, were made initially for light-scattering purposes by stirring the dry sample in solvent for at least 24 h. The resulting solutions were centrifuged at 31,000*g* in stainless-steel tubes in a preparative ultracentrifuge for 1–1.5 h at room temperature, and then filtered directly into a light-scattering cell through a Selas 02 filter. This procedure was unsatisfactory because the $\bar{M}_w$ values were 20–50% greater than those measured in water.

Suspected "micro gel" was identified by the following means. First, samples of potato amylose were dissolved in water and methyl sulfoxide (~0.5% (w/v) and then sedimented at an average of $\sim 6.3 \times 10^4 g$ (namely, 39,000 r.p.m.) in a Spinco Model E ultracentrifuge. The schlieren pattern peak of amylose in water moved towards the cell bottom at a rate of 0.24 mm/min, whereas in methyl sulfoxide it moved at a rate of 0.049 mm/min. As the amylose sedimented about 4.9 times faster in water than in methyl sulfoxide the in the preparative ultracentrifuge time was increased in an effort to clarify the methyl sulfoxide solutions thoroughly. Decrease in concentration, as determined by optical-rotation measurement on the clarified solution, was chosen as a measure of clarification efficiency. After 20 h at 25,000–30,000*g* in a Spinco model L centrifuge, the amylose solution in methyl sulfoxide showed: (*1*) no loss of concentration when the rotor was decelerated with the brake on, and (*2*) a loss of about 8% concentration when the rotor was decelerated without a brake. These results demonstrate that clarification of methyl sulfoxide solutions of amylose (and dextran) by the preparative ultracentrifuge methods commonly used is likely to be unsatisfactory.

In order to obtain consistent $\bar{M}_w$ values in solvents rich in methyl sulfoxide, the dried powder was dispersed and dissolved in boiling, distilled water and the aqueous solution was clarified as previously described. An equal volume of methyl sulfoxide was then added to the clarified, aqueous solution. This solution was distilled in glass under vacuum with stirring and gentle heating to remove the water, which distilled off when the distillate vapors reached 55–60° at about 1 torr. The resulting solution of amylose in methyl sulfoxide was filtered through a medium-porosity, glass frit and the concentration was determined by optical rotation. After the solution had been diluted to the desired concentration and solvent composition, it was refiltered through a Selas 02 filter directly into a light-scattering cell. This procedure yielded $\bar{M}_w$ values in methyl sulfoxide or 9:1 Me_2SO–H_2O that were within 5% of values measured in water. A flow diagram summarizes the results on preparation of an amylose solution.



Flow diagram showing methods used for solution preparation.

Specific rotations, $[\alpha]_D^{25}$, of samples in water were determined as previously described on a dry-weight basis of dissolved material remaining after solutions had been evaporated[7].

Dialysis for purposes of comparing dialyzed *vs.* nondialyzed $d\bar{n}/dc$ values was conducted at room temperature for at least 2 days in water and at least 5 days in methyl sulfoxide or Me_2SO–H_2O solvents. The solvent outside the dialyzing solutions was agitated by Teflon®-covered stirring-bars. Some samples of Pharmacia® dextran used in $d\bar{n}/dc$ measurements of nondialyzed solution–solvent pairs was taken directly from the bottle and vacuum dried, whereas other measurements were made on dextran that had first been dialyzed in regenerated cellulose tubing against distilled water, lyophilized, and then vacuum dried.

Dialysis experiments to quantitate preferential solvation were conducted for at least 14 days at 25° in a constant-temperature bath. These experiments were effected in closed Pyrex® tubes, which contained both solvents, and the Vycor® thimble, which contained solution. Gentle mechanical jarring provided movement to agitate liquids in the tubes. The quantity of solution within the porous-glass thimble, and solvent outside, was obtained by weight.

The composition of dialyzed solvent was determined in a differential refractometer by comparison with nondialyzed stock solvent of known composition. A series of calibrating solvents was made by adding measured weights of methyl sulfoxide to weighed amounts of stock solvent. A deflection, ΔD, was measured between stock solution and calibrating solution, and composition was obtained from a graph of ΔD *vs.* solvent composition. Data of Cowie and Toporowski[12] were used in converting solvent refractive index to solvent composition. Because this method is sensitive to whatever factors affect refractive index, $\bar{n}$, separate carbohydrate analyses by the phenol–sulfuric acid method[13] were performed on stock solvent and dialyzed solvent to determine whether or not carbohydrate had diffused through the porous glass.

RESULTS AND DISCUSSION

Molecular weight–intrinsic viscosity behavior of amylose in methyl sulfoxide. — Light-scattering results from aqueous solution, and intrinsic-viscosity measurements in methyl sulfoxide solutions on the fractions of corn and potato amylose are presented in Fig. 1. Included are lines that represent extremes of published values of Mark–Houwink–Sakurada equations. The data fall within the region formed by these extremes. Differences in methods of treating solute could cause a variation of up to 5% in measured values of $[\eta]$. Viscosities of four of the corn-amylose fractions were measured in 95% Me_2SO–5% H_2O and $[\eta]$ was corrected to 100% methyl sulfoxide by using data of Cowie[14]. This correction yielded up to a 5% increase in $[\eta]$. The slope of the corn-amylose data (Fig. 1) is about that of data obtained by Everett and Foster (———), whereas the potato-amylose data exhibit a slope similar to data of Cowie (—·—·—). Forcing a best-fit line through all of the points would yield a

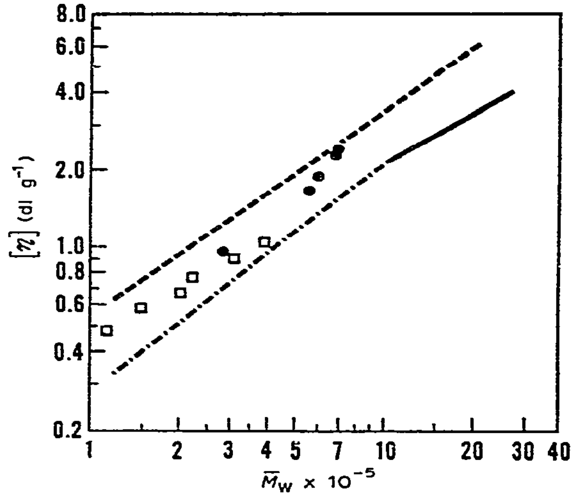


Fig. 1. Viscosity–molecular weight relationship of amylose in methyl sulfoxide. $\bar{M}_w$ values are those obtained in aqueous solution. □ corn amylose, this work; ● potato amylose, this work; — — — Burchard, ref. 4; — • — • — Cowie, ref. 5; ——— Everett and Foster, ref. 6.

function about parallel to, and of greater $\bar{M}_w$ values, than results of Fujii, Honda, and Fujita[2].

We consider it reasonable to suggest that the $\bar{M}_w$:[η] behavior shown in Fig. 1 represents actual differences in amyloses obtained from different botanical sources, rather than experimental scatter from a line common to all points. Two causes of possible botanical differences among natural amyloses that might affect viscosity behavior in methyl sulfoxide are variations in side-chain branching and variations in the content of such charged groups, as phosphate linkages in a potato-amylose, polymer backbone. Another possibility is that differences in the preparation and treatment of the starches from which the amyloses were extracted might have been sufficient to cause composition differences that would affect viscosity behavior in methyl sulfoxide. For whatever reasons, our results and those of others[2–6] indicate that natural amyloses to not behave identically.

It should be noted that the molecular weight of amylose ought not to be estimated by measurement of [η] in methyl sulfoxide on the basis of present literature values. For a measured [η] value, cumulative experimental error, differences in treatment of samples, and possible behavior variations reflecting differences in botanical source, may lead to uncertainties of 100% or greater in $\bar{M}_w$, within the region displayed in Fig. 1. (Effects of sample treatment upon viscosity–molecular weight reproducibility were discussed by Banks and Greenwood[15].) Problems in removing polysaccharide aggregates or "micro gel" from solutions in methyl sulfoxide suggest that $\bar{M}_w$ measurements may be more reliable when performed in aqueous solution.

Specific refractive increment behavior. — Effects upon $d\bar{n}/dc$ of dialyzing *vs.* not dialyzing in Me_2SO–H_2O solvent are displayed in Fig. 2. Dialyzed dextran samples (●) have distinctly different values than their nondialyzed, solution–solvent counterparts (*Δ*, +) when the refractive index is: $1.34 \leqq \bar{n} \leqq 1.44$. For $\bar{n} \geqq 1.44$ (high concentration of methyl sulfoxide), dialysis of dextran does not affect $d\bar{n}/dc$ within experimental error. Lyophilized dextran ("purified" first by dialysis against distilled water) (+), appears to yield slightly higher $d\bar{n}/dc$ values than dextran taken directly from the bottle (*Δ*). Dialyzed samples of potato amylose (not shown) exhibit the same values of $d\bar{n}/dc$ as dextran in the solvent composition range $\bar{n} > 1.45$. However, nondialyzed potato-amylose samples taken directly after 8 years of storage showed lower values (□).

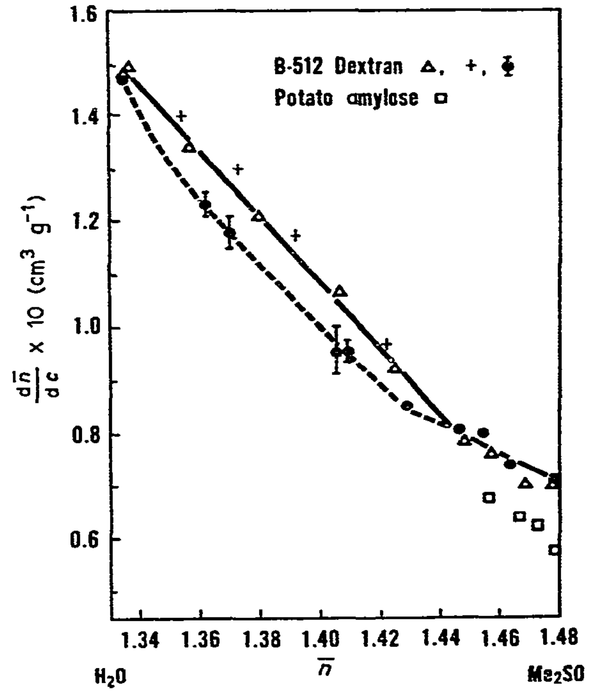


Fig. 2. Differential refractive measurements in Me_2SO–H_2O solvent at 25.0°. ● dextran, dialyzed; △ Dextran from bottle, not dialyzed; + dextran not dialyzed against Me_2SO–H_2O, but purified by dialysis against distilled water and lyophilized prior to use; □ potato amylose stored for 8 years, not dialyzed.

Concern that possible contamination might affect $d\bar{n}/dc$ led us to prepare two separate samples from the stored amylose. Portions of stored potato amylose were dissolved in boiling, distilled water, clarified, titrated with ethanol to precipitate all but the lowest molecular-weight fractions, and dried in a vacuum oven after treatment with ethyl ether as previously described. Measurements on nondialyzed solutions of these two samples yielded $d\bar{n}/dc$ values at given solvent compositions that were different from each other and greater than values obtained from the nondialyzed parent sample (□). Thus, the two fresh preparations exhibited functions of nondialyzed $d\bar{n}/dc$ *vs.* solvent compositions that would be represented as two lines roughly parallel to and between the values for dialyzed solution and the values (□) for nondialyzed, stored amylose solution. Measurements on amylose were not made when $\bar{n} < 1.44$ because retrogradation would occur at solvent compositions $< \sim 70\%$ of methyl sulfoxide (v/v).

Values of differential refractive index presented in Fig. 2 lead to several conclusions. The region of solvent composition in which dextran is preferentially solvated by water or water-enriched solvent complexes, $\sim 1.34 < \bar{n} < 1.44$, is indicated clearly by the two lines of dialyzed (●) and nondialyzed (Δ, +) $d\bar{n}/dc$ values. We postulate that dialyzed and nondialyzed $d\bar{n}/dc$ values are the same when $\bar{n} > 1.44$ because water is attracted by methyl sulfoxide to form the complex[12], $Me_2SO \cdot 2H_2O$, rather than by dextran. At solvent compositions of $\bar{n} > 1.44$, there is sufficient molar concentration of methyl sulfoxide to bind water that was previously bound by dextran. Our experience that dextran dissolves faster in water than in methyl sulfoxide is compatible with the concept that water is the better solvent for dextran.

Both dialyzed and nondialyzed B-512(F) dextran systems exhibit functions of $d\bar{n}/dc$ *vs.* solvent composition that are nonlinear (Fig. 2). Potato amylose must also show nonlinear behavior, as inferred from the observations that, for $\bar{n} > 1.45$, dialyzed $d\bar{n}/dc$ values are the same as for dextran and that for potato amylose, $d\bar{n}/dc$ in water $= 0.152$ cm^3/g. These measurements differ from work of Erlander and Tobin[16] who reported $d\bar{n}/dc$ values for dent corn amylopectin dialyzed in regenerated cellulose tubing to be a linear function of solvent composition in their Me_2SO–H_2O solutions of $\bar{n} > 1.40$. Our sample of potato amylose ,dialyzed in porous-glass thimbles, whose concentration was determined by optical rotation ($[\alpha]_D^{25}$, $+190°$ in methyl sulfoxide), exhibits a $d\bar{n}/dc$ value of 0.0695 ± 0.0020 at 546 nm, 25°. This is higher than the 0.0659 cm^3/g value reported by Everett and Foster[9] for amylose in methyl sulfoxide.

As mentioned previously in studies of the optical rotatory behavior of amylose[7], in the vicinity of $\bar{n} = 1.44$ it is possible that the polymer experiences a perturbation caused by a fairly abrupt change from a water-like to a methyl sulfoxide-like solvent environment. (Erlander has proposed specific mechanisms for such solvent interaction with starch[16].) This change may be expected to affect $d\bar{n}/dc$ behavior of dextran and that of amylose, too. Thus, H_2O–Me_2SO solvent interactions with polymer are demonstrated to affect optical rotation behavior of amylose and the $d\bar{n}/dc$ behavior of dextran.

Dangers of accepting a nondialyzed $d\bar{n}/dc$ measurement are demonstrated by the potato amylose data (□) and by the "purified" dextran (+) values, which are consistently greater than "from-the-bottle" values (Δ). We infer from these observations that small amounts of such matter as salts, degradation products produced during storage, or such solvents as alcohols, ether, acetone, or water may remain with "dried" material that is thought to be pure and so cause false $d\bar{n}/dc$ behavior if the solution is not dialyzed. As $d\bar{n}/dc$ appears as a squared term in the Rayleigh ratio, errors in $d\bar{n}/dc$ cause compounded errors in the calculated $\bar{M}_w$ value.

Preferential water solvation of dextran. — The notation of Casassa[17] is used to estimate an "absorption" coefficient, α, which is defined as $(\delta\phi_1/\delta C_2)_\mu$ and indicates preferential solvation between a dialyzing polymer, component 2, and one component of a binary solvent:

$$\left(\frac{\delta\bar{n}}{\delta C_2}\right)_\mu = \left(\frac{\delta\bar{n}}{\delta C_2}\right)_{\phi_1} + \alpha\frac{d\bar{n}}{d\phi_1} \qquad (1)$$

whereby values of $(\delta\bar{n}/\delta C_2)_\mu$, the differential refractive index at constant chemical potential, μ, and $(\delta\bar{n}/\delta C_2)_{\phi_1}$, the differential refractive index at constant volume fraction of one solvent component, ϕ_1, are taken from the dashed line and the solid line, respectively, of Fig. 2. Volume fraction of methyl sulfoxide, ϕ_1, is estimated by:

$$\phi_1 \sim \frac{\text{volume Me}_2\text{SO}}{\text{in 25 ml mixed solvent}}.$$

Fig. 3 shows results of plotting ϕ_1 as a function of $\bar{n}$. The slope of this function at a chosen $\bar{n}$ value is taken as $\sim d\bar{n}/d\phi_1$.

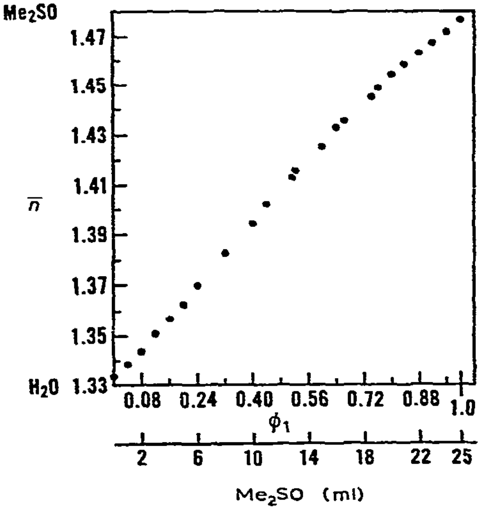


Fig. 3. Relation between refractive index and volume fraction of methyl sulfoxide, ϕ_1, in the solvent system $Me_2SO–H_2O$.

Table I presents the measurements and calculations. A negative value of α represents a decreased amount of methyl sulfoxide required in the polymer (namely,

TABLE I

SPECIFIC REFRACTIVE INCREMENTS AND ABSORPTION COEFFICIENT OF DEXTRAN IN METHYL SULFOXIDE–WATER[a]

$\left(\frac{\delta\bar{n}}{\delta C_2}\right)_\mu$	9.6×10^{-2}
$\left(\frac{\delta\bar{n}}{\delta C_2}\right)_{\phi_1}$	0.106
$\frac{d\bar{n}}{d\phi_1}$	0.155
$\alpha \equiv \left(\frac{\delta\phi_1}{\delta C_2}\right)_\mu$	-6×10^{-2}

[a] Solution $\bar{n} = 1.405$.

dextran) solution relative to the solvent mixture to maintain osmotic equilibrium in the dialyzing system.

A method of estimating the amount of additional water in the dextran solution is outlined here and in Table II. Composition of solvent outside the porous-glass thimble after dialysis is determined from a measured ΔD value and the calibration plot of ΔD *vs.* solvent composition in units of g Me_2SO/g solvent. It is assumed that

TABLE II

WATER ASSOCIATED WITH DEXTRAN

Run 2A[c]	g Me_2SO/g solvent
Stock solvent (ΔD = 0)	0.4850[b]
Solvent after dialysis (ΔD = 1.0936)	0.4882[c]
Wt. of solvent in dialysis chamber	8.62 g
Wt. of water insolvent before dialysis	$(1-0.4850)\ 8.62 \equiv A$
Wt. of water in solvent after dialysis	$(1-0.4882)\ 8.62 \equiv B$
Wt. of water that entered solution	$A-B = 0.028$ g
Wt. of solution in porous tube	4.26 g
Conc. of dextran in solution	2.30%
Wt. of dextran in solution	9.8×10^{-2} g
Wt. estimate	0.29 g H_2O/g dextran

Summary

Run no.	1	2[a]	2[d]	3[a]	3[d]	4[a]	4[d]
Water (g)/g dextran	0.28	0.21	0.21	0.29	0.28	0.40	0.30
Dextran in solution (μg/ml) $\times 10^{-3}$	32.5	20	20	23	23	20	20
Carbohydrate in solvent (μg/ml)	4			7	20		

Average of 7 values: 0.28 ±0.06 g H_2O/g dextran

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[a] Long tube. [b] Data from ref. 12. [c] Value from calibration graph. [d] Short tube.

there is no change in volumes and in weights of solvent and solution in the closed system during dialysis. Water entering the dextran solution to establish osmotic equilibrium averages 0.28 g water/g dextran. If the formula weight of a dextran backbone-sugar residue is estimated to be 160, a calculation shows that about 2.5 molecules of water preferentially enter the solution for every D-glucopyranose residue of dextran derived from microorganisms of type, NRRL B-512(F).

The porous thimbles did allow some carbohydrate to diffuse into the solvent chamber. However, the ratio $\dfrac{\text{concentration of dextran in solution}}{\text{concentration of carbohydrate in solvent}}$, is $>10^3$ (Table II). It seems that the relatively small amount of carbohydrate that diffused into the solvent is not a major source of variation in our results. Except for run 1, which was made in a porous tube that cracked upon subsequent handling, Table II presents data from three separate experiments run in duplicate in a "long" (*a*) and a "short" (*d*) porous Vycor® tube.

Stability of specific rotation. — Table III presents comparisons of specific rotations taken on fresh and aged samples of amylose by a variety of procedures. The sample of dent corn amylose remained stable. Previous and present values for animal-

TABLE III

SPECIFIC ROTATION

Sample	$[\alpha]_D^{25}$ *in water*				$[\alpha]_D^{25}$ *in methyl sulfoxide*			
	Previous	*No. of values*	*Present*	*No. of values*	*Previous*	*No. of values*	*Present*	*No. of values*
Potato amylose[a]	201	2[b]	192.8 ±2.2	3[b]	194	[c]	189.6 ±0.6	6[d]
			194.2	1[e]			189.3 ±0.6	3[f]
							188.2 ±0.7	3[g]
							180.7 ±0.7	5[h]
Dent-corn amylose[a]	197.4 ±1.5	5[b]	194.9 ±1.4	4[b]	190	[c]	188	2[d]
Animal-liver glycogen[i]	196	2[b]	194 ±3	3[b]	191	[c]	183.5	1[d]
							183 ±3	5[h]
Potato amylose[j]			195	2[b]			184.8	1[d]
							182.5	1[k]

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[a] Sample used in ref. 7 and stored for 8 years in glass at room temperature. [b] Values derived by evaporation of clarified aqueous solution. Previous values from Ref. 7. [c] Value obtained by extrapolation to 100% methyl sulfoxide by a dilution method based on the previous $[\alpha]_D^{25}$ in water (Ref. 7). [d] Dry-weight basis of sample freshly precipitated from clarified aqueous solution as ethanol complex, washed with diethyl ether, vacuum dried at 90°, and stored without exposure to moisture. [e] Sample dissolved in boiling water, clarified, precipitated as ethanol complex, washed with diethyl ether, vacuum dried, and then treated as in *b*. [f] Treated as *d*, and exposed to air for 2 days. [g] Treated as *d* and exposed to air for 4 days and then stored for 2 weeks. [h] Dry-weight basis of sample that had been exposed to moisture and stored for 8 years. Standard deviations are indicated when 3 or more independent measurements were made. [i] History of this sample uncertain. [j] Sample freshly extracted from potatoes at this Center. [k] Treated as *g* and stored for a total of 6 weeks.

liver glycogen should not be compared directly, because the previous and present samples may not have been the same. It may be noted that the greatest standard deviation in $[\alpha]_D$ for given sample and conditions is ~1.7% for glycogen in methyl sulfoxide and ~1.2% for potato amylose in water (aged sample). We observed that the $[\alpha]_D$ in water for potato amylose decreased slightly with age ,from 201 to about 194. This decrease also is reflected in $[\alpha]_D$ (methyl sulfoxide) changing from 194 to 189.6. Effects of treatment upon the aged, potato amylose sample can significantly alter $[\alpha]_D$ (methyl sulfoxide), which increased from about 181 to 189 when the sample was redissolved in boiling, distilled water, predcipitated as the ethanol complex and suitably dried.

Exposure of vacuum-dried samples to moisture in the air for short periods of time did not significantly after the $[\alpha]_D$ (methyl sulfoxide) values of samples in Table III when the proper moisture correction was made. A final point from Table III is that whereas the $[\alpha]_D$ (water) values for the two potato amylose samples are within experimental error, the $[\alpha]_D$ (methyl sulfoxide) appears to be slightly higher (189.6) for the aged sample reprecipitated from water than for the fresh, potato-amylose sample (185).

CONCLUSIONS

We end this study with the strong suspicion that: (*a*) cumulative experimental error in measurements such as these may be greater than often realized, and (*b*) that actual differences in amyloses, whether arising from variation in botanical source or treatment of samples, may explain part of the lack of agreement over $\bar{M}_w:[\eta]$ behavior in methyl sulfoxide. The advantages of using a dialyzed solution–solvent pair to obtain $d\bar{n}/dc$ values free of errors caused by potential impurities and preferential solvation troubles appear most convincing. B-512(F) Dextran has been shown to associate with water over a major portion of the mixed solvent-system, Me_2SO–H_2O. The information provided here should be of use to researchers concerned with solution properties of other polysaccharides in dimethyl sulfoxide-containing solvents.

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